SOLUBLE BRANCHED POLYMERS OF GLUCOSE AND PROCESS FOR PRODUCTION THEREOF

The invention concerns soluble branched polymers of glucose containing essentially no β glucosidic bonds, having particular contents of α-1,6 glucosidic bonds, excellent stability in solution expressed by their low tendency to retrograde and a remarkable molecular weight distribution in a range lying between 10^4 and 10^8 daltons.

These soluble branched polymers of glucose furthermore have a low reducing sugar content and low viscosity.

The invention also concerns a process for manufacture of said soluble branched polymers of glucose. It also relates to compositions containing such soluble branched polymers of glucose which it is possible to use in many industrial applications and particularly in the food industries.

In the sense of the invention, the soluble branched polymers of glucose containing essentially no β-glucosidic bonds are polymers of α-1,4 linked glucose and exhibit many α-1,6 ramification points (also called branching points), and less than 5% of β-branching, that is to say β-1,2, β-1,3, β-1,4 or β-1,6, branching.

The glucose polymers normally industrially accessible are in particular derived from natural or hybrid starches and derivatives thereof.

Generally, starch is made up of two polymers, amylose and amylopectin. Amylose is the fraction containing linear α-1,4 linked homopolymers of glucose and some α-1,6 branching points.

Amylopectin is the ramified fraction, made up of linear α-1,4 chains of glucose linked to other linear α-1,4 chains of glucose by α-1,6 ramification points.

The combination of these two homopolymers, packaged in the form of very well structured granules of starch, constitutes the carbon source reserve of the plant.

The starch produced in each plant is made up of a variable percentage of each of its constituents amylose and amylopectin, or even a particular distribution of the molecular weights of each of said homopolymers of glucose. This explains the reason why the various starches and derivatives thereof are usually classified on the basis of their botanical origin.

Moreover, the functional properties of starches and derivatives thereof are directly dependent on their content of amylose and amylopectin. Thus, when a suspension of starch is heated above its gelatinization temperature, the starch granule swells, and the amylose solubilizes preferentially. However, on cooling of the suspension, the homopolymers of glucose retrograde, rapidly for amylose (a few hours) and more slowly for amylopectin (a few days).

Specialists in the field of the utilization of starches and derivatives thereof in the food industry, concur in stating that this phenomenon of retrogradation affects the texture of foodstuffs, and diminishes their lifetime.

It is known that these products are rendered more acceptable by preparing them from starchy products rich in amylopectin, and thus for example from waxy varieties. However, the stability of the gels and binders obtained from said starchy products rich in amylopectin is not sufficient for the requirements of the food industries, where it is sometimes necessary to have a storage life of several months.

A first solution consists in stabilizing the glucose homopolymers by means of chemical agents. This operation is mostly effected by the use of esterification or etherification reactions. These can in particular be acetylation or hydroxy-propylation reactions. Further, to obtain the desired properties of texture and viscosity, these reactions are often combined with a crosslinking reaction.

These modifications then confer outstanding rheological properties on the starches, rendering them more resistant to mechanical processes such as shear, or to acidic media. Acetylation or hydroxypropylation further confer good storage stability after cooking, particularly at low temperature.

However, the products thus obtained have the disadvantage of having been treated chemically, which is often unfavorably viewed by consumers.

A second solution consists in isolating the starch from plants certain of whose genes involved in the biosynthesis of the starch have been altered, which confers particular properties on the starches thus modified.

These can be mutant or hybrid varieties, affected at the level of the waxy (wx), amylose extender (ae), dull (du), opaque (o), shrunken (sh), brittle (bt) or sugary (su) genes.

Thus patent 4,767,849 describes the starch extracted from a maize variety homozygotic for the genotype waxy/shrunken-1, which confers on the granular starches thus obtained properties of stability to retrogradation in deep-freeze/defrost cycles (usually called freeze/thaw cycles) equivalent to the chemically modified starches. However, these varieties obtained by crossing between two varieties of waxy and shrunken genotype only have a starch content lying between 1 and 20% of the starch content normally synthesized by so-called wild type varieties.

They can also be genetically modified plants, obtained by targeted modification of a gene or of a group of genes coding for enzymes involved in the biosynthesis of starch. The strategies for gene extinction or amplification in the plant, genes coding for example for the starch debranching or branching enzymes proper to the plant, or of exogenous origin, such as the glycogen biosynthesis genes of bacteria, have been abundantly described.

However, it has to be said, as in the case of mutant or hybrid plants, that if the starches thus modified have properties equivalent to the chemically modified starches, the starch contents of the plants thus obtained are far from being industrially satisfactory.

A first alternative to these processes consists in utilizing enzymes of the α-amylase, α-amylase,

pullulanase or iso-amylase type to modify native starches *in vitro* in order to confer on them certain of the properties of the chemically modified starches. There are thus normally no longer problems connected with the quantities used.

Thus patent application EP 539,910 describes a process for preparation of granules of starch modified by an α-amylase treatment to obtain products of lower viscosity. However, this process aims only to alter the structure of the starch granule, without profoundly modifying its constituents.

Patent EP 574,721 describes the preparation of a starchy product with a high content of stable amylopectin, by using no chemical treatment as such, but by carrying out a controlled hydrolysis reaction with β-amylase on a native granular starch.

The product thus prepared then displays an absence of syneresis and of viscosity change with time and is stable to freezing/thawing. However, this process necessitates a prior heat treatment, at a temperature lying between 65 and 75°C, to gelatinize the starch before performing the enzymatic hydrolysis as such. Moreover, it is necessary above all to control the hydrolysis level to limit it to a value lying between 5 and 20%.

Another alternative to processes aiming to modify native starches chemically, or to extract native starches having properties of modified starches from mutant, hybrid or genetically modified plants, consists in introducing new branching points into the starch *in vitro*.

This then involves performing a modification of the amylopectin or amylose chains, rather than using stabilization and/or crosslinking reactions as indicated previously.

Two techniques are normally utilized. The first uses thermal means, and the second purified enzymes for biosynthesis of glycogen and/or starch, such as glycogen or starch branching enzymes, respectively responsible for the synthesis of the α-1,6 branching points of glycogen or α-1,6 branching points of amylopectin, and of some branching points of amylose.

Patent application WO 95/22562 describes, for exemple, dextrins of the starch type, characterized by their molecular weight lying between 15 x 10^3 and 10^7 daltons, and a degree of branching lying between 2 and 8%, obtained by the treatment of native granular starch, in particular potato starch, under acidic conditions (orthophosphoric acid 0.17% by weight of starch) and at a temperature lying between 110 to 140°C for 1 to 15 hours.

The composition thus obtained is intended for sportspersons as an energy supply after physical effort. However, this treatment is long and very laborious to implement, and it leads to glucose polymers which contain, apart from a high content of α-1,6 bonds (preferably lying between 3 and 7%), new types of bonds which do not normally exist in native starch. In fact, nuclear magnetic resonance (NMR) analyses reveal bonds of the β-1,4 and β-1,6 type and α bonds other than α-1,4 and α-1,6.

From all of the foregoing, it emerges that there therefore is an unsatisfied need for having available,

firstly, glucose polymers having outstanding properties, in particular in terms of stability, solubility and possibly viscosity and by the same token conferring improved lifetime and digestibility properties on the products that contain them, and secondly, for obtaining them without using chemical or physical techniques, nor having recourse to extraction from mutant or genetically modified plants.

The Applicant company has succeeded in reconciling all these objectives hitherto considered difficult to reconcile, by imagining and developing, by dint of much research, novel types of products, namely novel soluble branched polymers of glucose containing essentially no β glucosidic bonds.

The soluble branched polymers of glucose containing essentially no β glucosidic bonds according to the invention are thus characterized in that they have between 2.5 and 10% of α-1,6 glucosidic bonds, a very low or zero tendency to retrograde in aqueous solution, determined according to a test A and a Mw determined according to a test C at a median value of the molecular weight distribution profile lying between 10^4 and 10^8 daltons.

The branched polymers of glucose according to the invention also have a low reducing sugar content, of at most 9% and a viscosity determined according to a test B, for 3 g of dry substance, of at most 5,000 cP.

The content of α-1,6 glucosidic bonds in the soluble branched polymers of glucose according to the invention, determined by proton NMR analysis, is from 2.5 to 10%, expressed as the number of α-1,6 bonds relative to the

total number of α-1,4 and α-1,6 glucosidic bonds in said branched polymers of glucose.

This content of α-1,6 glucosidic bonds confers on any glucose polymer according to the invention a particular structure, in terms of degree of ramification and/or lengths of ramified chains compared to the starch or starch derivative from which it is derived.

The soluble branched polymers of glucose according to the invention also display a low tendency to retrograde in aqueous solution, determined according to a test A. This test consists in establishing the susceptibility of a given product to retrogradation in the course of repeated freeze/thaw cycles.

The observed retrogradation of the product, and the enthalpy of destructuring of the product which was able to retrograde, determined by differential calorimetric analysis, thus provide information on the stability of the product under consideration.

More precisely, test A consists in making an aqueous preparation of the product to be tested having 40% dry matter. Different samplings are made in hermetically closed crucibles. All of the crucibles are heated to a temperature of 100°C for 15 mins to effect the gelatinization or dissolution, and these crucibles are then subjected to a treatment of freeze/thaw cycles, each of the cycles consisting in bringing and maintaining said preparation for 15 mins to a temperature of -20°C, then to a temperature of 20°C and in then maintaining it at that temperature for 1 hr 30.

A differential calorimetric analysis is then performed in each cycle, on Perkin Elmer equipment, for

the determination of the enthalpy of destructuring of the product which has then been able to retrograde.

The stability to freeze/thaw cycles is thus estimated firstly by the number of freeze/thaw cycles beyond which this measurement of the enthalpy value required to destructure the starch gel which has then retrograded can be performed.

The glucose polymers according to the invention subjected to these repeated freeze/thaw cycles, display, surprisingly and unexpectedly, a "low tendency to retrograde", that is to say here a partial, or even total absence of retrogradation according to test A and dependent on their content of α-1,6 glucosidic bonds.

Thus the glucose polymers according to the invention which have a content of α-1,6 glucosidic bonds lying between 2.5 and 5%, only start to retrograde significantly beyond the eighth freeze/thaw cycle, displaying a low retrogradation enthalpy value, as will hereinafter be exemplified.

They are described as branched polymers of glucose displaying "a very low tendency to retrograde".

As for the glucose polymers according to the invention which have a content of α-1,6 glucosidic bonds lying between 5 and 10%, no retrogradation of the solution is observed even after 12 freeze/thaw cycles, which explains why no enthalpy of destructuring can be established.

It is particularly surprising that the glucose polymers according to the invention can present such stability. In fact, the measurements made with test A on waxy starches and crosslinked and acetylated waxy

starches (such as those prepared following the teachings of US patent 2,928,828) retrograde between the fourth and the sixth freeze/thaw cycle, as will be shown in example 2.

Thus, to the knowledge of the Applicant company, no glucose polymers which have such stability exist.

This property quite naturally renders the branched glucose polymers according to the invention suitable for compositions utilizable in the food industry, which then have high storage stabilities.

Another advantage of the invention is that of making it possible to obtain a finished product, utilizable for example as an instant binder in refrigerated or deep-frozen products.

The determination of the median value of the molecular weight distribution profile of the soluble branched polymers of glucose according to the invention is performed by measurement of the weight average molecular weight (Mw).

In practice, the Mw values are not calculated, but are measured by various techniques. For example, a measurement method suitable for glucose polymers is used, which is based on gel permeation chromatography on chromatography columns standardized with pullulans of known molecular weights.

Test C, developed by the Applicant company to determine the median value of the molecular weight distribution profile characteristic of the soluble branched polymers of glucose according to the invention consists:

- in establishing the molar distribution profile of the chromatographic fractions of said soluble branched polymers of glucose,
- in determining the value called "median value of molecular weight distribution profile" which corresponds to the value of the mean molecular weight distribution peak of the population representing more than 90% of the chromatographic fractions derived from said separative gel permeation chromatography.

The branched polymers of glucose according to the invention then have an adjusted molecular weight distribution profile value Mw lying between 10^4 and 10^9 daltons.

Advantageously, the soluble branched polymers of glucose according to the invention can be classified into two families, the first family having a median Mw value of the molecular weight distribution profile lying between 10^5 and 10^6 daltons and the second family having a median Mw value of the molecular weight distribution profile lying between 10^7 and 10^8 daltons.

In addition, the soluble branched polymers of glucose according to the invention have a low reducing sugar content.

The determination of the reducing power of the branched polymers of glucose according to the invention, by any method known to the skilled person, leads to values of at most 9%.

Advantageously, the branched polymers of glucose can be classified into two subfamilies on the basis of their reducing sugar content.

The first subfamily has a reducing sugar content of at most 1%.

The second subfamily has a reducing sugar content lying between 5.5 and at most 9%.

The Applicant company has further found that the branched polymers of glucose according to the invention have quite exceptional rheological properties.

The viscosity analysis of the branched polymers of glucose according to the invention is performed by means of a test B developed by the Applicant company for this particular range of products.

These are in fact not granular products such as are usually described and analyzed in the prior art, but branched polymers of glucose which surprisingly and unexpectedly display outstanding solubility in cold water.

Test B consists in firstly preparing the product to be analyzed by precipitation with ethanol, drying under vacuum, then grinding in the mortar, and finally screening on a 125 µm mesh. A mass of between 3 and 15 g of the dry product to be analyzed thus obtained is then introduced, with 6.75 g of 98% purity glycerol, into the bowl of a Rapid Visco Analyzer (RVA - NewPort Scientific), and the whole is carefully homogenized using a microspatula.

A quantity of demineralized water is next added, in order to obtain a final mass of 28 g. The whole is then immediately stirred. The time/temperature and speed analysis profile in the RVA is then performed as follows. The sample is stirred at 100 rpm at a temperature of 25°C for 5 secs, then at 500 rpm for 25 secs. The stirring is

then maintained at 160 rpm during the rest of the profile. The initial temperature of 25°C is maintained for 10 mins, then it is increased to 90°C in 8 mins. This temperature of 90°C is then maintained for 3 mins, decreased to 30°C in 8 mins, then maintained at this value of 30°C for 5 mins.

The viscosity retained is the viscosity in centipoises (cP) measured at the end of the analysis profile, at 34 mins.

The branched polymers of glucose according to the invention then have a viscosity of at most 5,000 cP for 3 g dry product.

The Applicant company has also found that these viscosity values of the branched polymers of glucose according to the invention are of the same order of magnitude as the viscosity values, determined following the same test B, of waxy starches fluidified by acid treatment.

However, supplementary viscosity measurement analyses carried out after seven days of storage at 4°C showed, surprisingly and unexpectedly, an outstanding stability of the viscosity of the branched polymers of glucose, in contrast to said fluidified waxy starches of the same viscosity, as will hereinafter be exemplified.

These products can therefore for example advantageously be used for the production of instant liquid food preparations, and above all make it possible to guarantee long term storage at low temperature.

The branched polymers of glucose according to the invention are thus particularly well suited for compositions intended for use especially in the Paper-

Cardboard, Textiles, Pharmaceutical, Cosmetics, and in particular Food industries.

To prepare the soluble branched polymers of glucose according to the invention, the sequence of the following stages is performed, consisting in that:

a) an aqueous suspension of starch or of starch derivative with dry matter of at least 1% by weight, preferably 2 to 50% by weight, is subjected to a temperature greater than 130°C, preferably lying between 140 and 150°C, under a pressure of more than 3.5 bars, preferably lying between 4 and 5 bars for at least 2 minutes, preferably for 2 to 5 minutes,

b) the starch thus obtained is treated with 50 to 2,000 units of purified branching enzyme at a temperature lying between 25 and 50°C, preferably at a temperature of 30°C for a period of 10 mins to 24 hours, and

c) the branched polymers of glucose thus obtained are collected.

The starch is introduced in aqueous solution with at least 1% by weight, preferably from 2 to 50% by weight, dry matter.

The choice of a source or of a quality of starch or of particular derivatives thereof is only of relative importance.

The Applicant company has found that the branched polymers of glucose according to the invention are easily synthesizable from starches or from derivatives thereof which already have a branching ratio of at least 1%.

This suspension of starches or of derivatives of starch is next subjected to a particular cooking treatment, which consists in treating it at a temperature

greater than 130°C, preferably lying between 140 and 150°C, under a pressure of more than 3.5 bars, preferably lying between 4 and 5 bars for at least 2 minutes, preferably for 2 to 5 minutes. This treatment is advantageously performed in a double-jacket tubular boiler heated with a heat-transfer fluid, equipment which it is easy for the skilled person to obtain.

The second stage of the process according to the invention consists in treating the starch thus obtained with 50 to 2,000 units of purified branching enzyme at a temperature lying between 25 and 50°C, preferably at a temperature of 30°C for a period of 10 mins to 24 hours.

The branching enzymes are selected from the group consisting of glycogen branching enzymes and starch branching enzymes. More preferably, the glycogen branching enzyme of *Escherichia coli*, and the starch branching enzymes, are chosen, and still more preferably the type I and type II starch branching enzymes of maize, or of unicellular algal starch, for example those of the green algae *Chlamydomonas reinhardtii*.

The isolation of the said glycogen or starch branching enzymes can be effected by any method in itself known to the skilled person.

Concerning the branching enzymes of unicellular algae, however, the Applicant company recommends utilization of the preparation process described in the French patent application filed under the No. 98/12051, of which it is the proprietor.

Access to the purified enzymes can be achieved from the mixture of algal enzymes thus obtained, by directly applying chromatographic separation techniques in

themselves known, or by the use of recombinant DNA techniques.

It can in fact be advantageous to prefer to isolate and express the genes coding for the unicellular algal starch branching enzymes in a microorganism more easily manipulable than the unicellular algae.

The technique, in itself known to the skilled person, then consists for example in:

- producing polyclonal antibodies specific for each of the algal branching enzymes previously purified,
- screening, with said specific antibodies, an expression bank of genomic DNA from the unicellular algae under consideration,
- isolating DNA fragments from the clones of said expression bank of genomic DNA which have reacted with one and/or other of the specific polyclonal antibodies,
- introducing said DNA fragments corresponding to the genes coding for the unicellular algal starch branching enzymes into bacteria allowing their expression.

The algal starch branching enzymes produced by this process are called recombinant branching enzymes, because derived from a unicellular alga, then transferred genetically and expressed in a microorganism of another species, in the present case a bacterium.

To prepare the soluble branched polymers of glucose according to the invention, a purified recombinant algal starch branching enzyme can then advantageously be made to act upon a maize waxy starch paste prepared according to stage a) of said process.

The last stage of the process according to the invention then consists in collecting the branched polymers of glucose thus obtained.

The products are precipitated by 3 volumes of ethanol, purified and dried under vacuum for 24 hours, or else atomized, by any technique otherwise known to the skilled person.

Other characteristics and advantages of the invention will appear on reading the non-limiting examples hereinafter described.

EXAMPLE 1

The preparation of the branched polymers of glucose is effected as follows. A suspension of waxy maize starch with a dry matter content of 2.5% by weight is prepared. This suspension is then treated in a laboratory tubular double-jacket boiler heated with heat transfer fluid, at a temperature of 145°C, under a pressure of 4 bars. The feed rate is 40 ml/min, for a residence time of 3 minutes in the said boiler.

1.5 liters of this preparation are cooled to ambient temperature and placed in a medium buffered to pH 7 with 0.1 M final Tris HCl buffer for a total volume of 3.750 liters. 19 ml (of an enzyme solution containing 1.8 mg/ml of proteins, moreover having a specific activity of 1,100 U/mg, activity measured by the phosphorylase A estimation method in itself known to the skilled person) of a solution of previously purified recombinant starch branching enzymes from the alga *Chlamydomonas reinhardtii* are added, and this is allowed to act at 30°C for 30 mins to obtain branched polymers of glucose according to the invention having an α-1,6 glucosidic bond content of 4.3%

(product A), and for 2 hours, to obtain branched polymers of glucose according to the invention, having an α-1,6 glucosidic bond content of 6% (product B). Each of the products is then precipitated with ethanol, filtered, rinsed and dried under vacuum for 24 hours.

The respective values of the median Mw of the molecular weight distribution profile of the products A and B are respectively 1.5 x 10^7 daltons and 2.2 x 10^7 daltons. Their reducing sugar contents are respectively 0.05% and 0.07%.

EXAMPLE 2

The determination of the stability of the branched polymers of glucose according to the invention is performed by measurement of the enthalpy of destructuring of the retrograded product, if there is a retrograded product, by differential calorimetric analysis, during repeated freeze/thaw cycles.

Two branched polymers of glucose according to the invention, having respectively an α-1,6 glucosidic bond content of the order of 4.3% (product A) and of the order of 6% (product B) are prepared as indicated in Example 1. The analysis is also effected on two other samples: waxy maize starch (product C) and a crosslinked and acetylated waxy starch having an acetyl index of 1.8 (product D).

As indicated in test A, an aqueous preparation of each of the 4 samples with 40% dry matter placed in a group of hermetically closed crucibles is made, and these are heated for 15 mins at 100°C in a Perkin Elmer DSC4 oven. For each crucible, 2, 4, 6, 8, 10 or 12 successive freeze/thaw cycles are performed according to the following protocol: 15 mins at -22°C, then 1 hr 30 at

20°C. A retrogradation enthalpy measurement is performed on each crucible by placing it in the Perkin Elmer differential calorimeter.

Table I below shows the retrogradation enthalpy measurements determined for each of the 4 products tested in the course of 12 successive freeze/thaw cycles.

Table I

Determination of retrogradation enthalpies during 12 freeze/thaw cycles, expressed in J/g of preparation.

PRODUCTS	Cycle 2	Cycle 4	Cycle 6	Cycle 8	Cycle 12
A	0	0	0	0	0.2
B	0	0	0	0	0
C	0	0	0.4	1	2.2
D	0	0.10	0.35	0.6	1.75

The branched polymers of glucose thus display outstanding stability, even after 12 freeze/thaw cycles. While the waxy starch (product C) and the crosslinked and acetylated waxy starch (product D) start to retrograde from the 4th freeze/thaw cycle, the same does not apply to each of the branched polymers of glucose according to the invention prepared from said waxy starch. The enzymatic procedure utilized to modify the starches and starch derivatives thus makes it possible to ensure for them excellent stability, as they stand much superior to the stabilized and/or crosslinked waxy starches.

EXAMPLE 3

The rheological characterization of the branched polymers of glucose according to the invention is effected using a Rapid Visco Analyzer (RVA).

The products according to the invention display outstanding solubility in cold water.

It was therefore necessary to develop a viscosity determination method appropriate for this type of product.

As indicated in test B, 4.5 g of the dry product to be tested are mixed with glycerol and water to reach a final mass of 28 g.

The products analyzed are firstly the products A, B and C described in example 2 and two other products E and F, corresponding to waxy maize starches fluidified to two levels of fluidification (value estimated by the standard measure of fluidity in water, i.e. the index of "water fluidity" or WF), obtained by treatment under acidic conditions in themselves known to the skilled person, the product E having a WF of 50, and the product F a WF of 65.

The time/temperature and speed analysis profile in the RVA is then performed as follows. The sample is stirred at 100 rpm at a temperature of 25°C for 5 secs, then at 500 rpm for 25 secs. The stirring is then maintained at 160 rpm during the rest of the profile.

The initial temperature of 25°C is maintained for 10 mins, then it is increased to 90°C in 8 mins.

This temperature of 90°C is then maintained for 3 mins, decreased to 30°C in 8 mins, then maintained at this value of 30°C for 5 mins.

Table II below shows the viscosity results for products A, B, C, E and F, expressed in centipoises.

Table II

Determination of viscosities at end of time/temperature and speed profiles in RVA of the products A, B, C, E and F, expressed in centipoises (cP)

PRODUCTS	Viscosity at 34 mins
A	1600
B	750
C	6060
E	1140
F	660

The branched polymers of glucose according to the invention still display some viscosity, but remarkable lower than that of the control waxy starch (C).

It can be seen that these viscosity values are of the same order of magnitude as the fluidified waxy starches.

A supplementary study is performed by measurement of the viscosity after storage for 7 days at 4°C.

This study makes it possible to characterize the stability of the pastes thus produced with time, and to determine how the branched polymers of glucose according to the invention differ from fluidified waxy starches.

The RVA bowls containing each of the five products are stored at 4°C.

The viscosity is then again determined by RVA. The time/temperature and speed profile is then characterized

by a speed and a temperature maintained respectively at 160 and 30°C for 20 mins.

The viscosity retained is the mean viscosity in cP measured between 15 and 20 mins.

Table III below shows the viscosity results obtained after 7 days of storage of the products A, B, C, E and F at 4°C.

Table III

Determination of the viscosity of the products after storage for 7 days at 4°C, expressed in cP.

PRODUCTS	Viscosity after 7 days at 4°C
A	2500
B	850
C	8650
E	white, hard, firm gel*
F	white, hard, firm gel*

* : viscosity not measurable

The results clearly show that the branched polymers of glucose according to the invention display outstandingly stable viscosity even after storage at 4°C. This low viscosity can therefore be advantageously exploited for food preparations which necessitate that the starchy ingredients which comprise them be of low viscosity (such as instant liquid preparations) and which have to be stored for a long period of time at low temperatures.

EXAMPLE 4

Soluble branched polymers of glucose according to the invention are prepared by causing a glycogen branching enzyme isolated from *E. coli* to act upon various solutions of starches and starch derivatives for 21 hours of reaction at 30°C and according to the other conditions described in Example 1.

In the present case, these are suspensions of standard maize starch (G), waxy maize starch (I), amylose-rich starch marketed by the Applicant company under the name of EURYLON® 7 (K) and a maltodextrin marketed by the Applicant company under the name of GLUCIDEX® 2 (M).

Table IV below shows the results obtained in terms of α-1,6 glucosidic bond content, value of the median Mw of the molecular weight distribution profile, reducing sugar content and retro-gradation behavior after 10 freeze/thaw cycles.

Table IV

Determination of the physico-chemical and functional characteristics of the soluble polymers of glucose according to the invention H, J, L and N obtained by the action of the glycogen branching enzyme of *E. coli* on the substrates G, I, K and M respectively with a given dry matter content.

	G 10%DM	H	I 1% DM	J	K 5% DM	L	M 20%DM	N
α-1,6 glucosidic bond content (%)	3	3.4	4.4	5.6	1.9	3.3	6.1	7.1
Centered Mw value (daltons)	5×10^7	5.8×10^5	1×10^8	2.2×10^5	8.5×10^6	5×10^5	3.3×10^5	1.4×10^5
Reducing sugar content	0.13	0.16	< 0.5	< 0.05	0.5	0.5	3	3.5
Enthalpies of retrogradation (J/g)	2	1	1.5	0	3	0.4	2.3	0

The soluble branched polymers of glucose according to the invention thus display outstanding freeze/thaw performance and an adjusted molecular weight distribution over a fine interval of values lying between 1.4 and 5.8 x 10^5 daltons, whereas the starting substrates on the contrary display a strong tendency to retrograde and molecular weight distribution profiles ranging from 10^3 to 10^8 daltons.